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EFFECTS OF SUBZERO TEMPERATURES AND TRAWLING STRESS ON SERUM OSMOLALITY IN THE WINTER FLOUNDER *PSEUDOPLEURONECTES AMERICANUS*

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Since the freezing point of sea water (–1.8° C) is approximately one degree Centigrade lower than the freezing point of the serum of most marine teleosts (–0.7° C to –0.8° C), the possibility exists that these fish may encounter temperatures that would freeze their blood. Most arctic (Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957; Eliassen, Leivestad and Møller, 1960; Gordon, Amdur and Scholander, 1962; Leivestad, 1965), Antarctic (DeVries and Wohlschlag, 1969; R. N. Smith, personal communication) and temperate zone (Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957; Umminger, 1969a, b) fish avoid freezing at subzero temperatures by existing in a supercooled state. However, a few species of Antarctic fish of the genus *Trematomus* have been reported to lower the freezing point of their plasma to make it isosmotic with sea water (DeVries and Wohlschlag, 1969; Potts and Morris, 1968). Pearcy (1961) has also reported that the winter flounder, *Pseudopleuronectes americanus*, forms an "antifreeze" at subzero temperatures by lowering the freezing point of its serum. These data on supercooling and "antifreeze" formation in fish are summarized in Table III. Since *P. americanus* is the only marine teleost from the temperate zone that has been reported to form an "antifreeze' in winter, the present investigation was undertaken in an attempt to confirm these observations.

Materials and Methods

The studies on temperature acclimation presented in this paper utilized laboratory-acclimated fish. Ten adult specimens of *P. americanus* were caught by trawl in Long Island Sound near Stonington, Connecticut, on January 3, 1967. Four of these fish were subsequently maintained in the laboratory on a photoperiod of eight hours of light per day at 15° C for five weeks; one fish was kept at 4° C for five weeks; three fish were kept at 4° C for four weeks and at –1.0° C for seven days; and one fish was maintained at 4° C for four weeks and at –1.5° C for seven days. The tenth fish was acclimated to 4° C for four weeks and transferred to water at –1° C; the temperature was gradually lowered to –1.8° C and the fish died. At autopsy, the tails of flounder lightly anesthetized with tricaine methanesulfonate (MS 222) were severed and the free-flowing blood was collected from the caudal artery. After centrifugation of the clotted blood, the serum was frozen at –20° C for later determinations of serum osmolality.

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TABLE I

Effect of temperature on serum osmolality of P. americanus

Temperature of acclimation	Fish no.	Sex	Serum osmolality (mOsm/liter)	Serum freezing point	Amount serum supercooled
15° C	1	M	373	−0.69° C	0° C
	2	M	423	−0.79° C	0° C
	3	M	403	−0.75° C	0° C
	4	M	355	−0.66° C	0° C
	Mean ± S.E.		389 ± 15	−0.72° C ± 0.02° C	
4° C	5	M	354	−0.66° C	0° C
−1.0° C	6	F	405	−0.75° C	0.25° C
	7	F	356	−0.66° C	0.34° C
	8	F	397	−0.73° C	0.27° C
	Mean ± S.E		386 ± 15	−0.71° C ± 0.02° C	0.29° C ± 0.02° C
−1.5° C	9	M	420	−0.78° C	0.72° C

Total osomolality, in reference to standard sodium chloride solutions, was estimated with a Mechrolab (new Hewlett-Packard) vapor-pressure osmometer equipped with Hamilton microsyringes. Statistical significance was determined using Student's t test.

In addition to the experiments on thermal acclimation, an investigation into the effects of trawling on serum osmolality was undertaken using ten freshly caught specimens of *P. americanus*. These fish were captured by trawl in water at 15° C from Long Island Sound near Old Saybrook, Connecticut, on October 14, 1966. The freshly caught fish were transported to the Essex Marine Laboratory in large steel wash buckets and autopsied at 15-minute intervals. Serum osmolality was determined (using the methods described above) for five fish killed 1–2 hours after capture and for five fish killed 5–6 hours after capture. There was no period of laboratory acclimation for these fish.

TABLE II

Effect of time after capture with trawl on serum osmolality of P. americanus

Group	Minutes after capture	Serum osmolality	Sex
Killed about 1–2 hours after capture	60	400	M
	75	392	M
	90	417	F
	105	412	F
	120	440	F
	Mean ± S.E.	412 ± 8	
Killed about 5–6 hours after capture	285	447	M
	300	448	M
	315	421	F
	330	531	F
	345	460	M
	Mean ± S.E.	461 ± 18*	

* Significantly different from group killed 1–2 hours after capture ($P < 0.05$).

Table III

Supercooling and "antifreeze" formation in marine teleosts

Family and species	Water temperature	Freezing point of serum	Amount serum supercooled	Reference
FAMILY ANARHICHADIDAE				
Anarhichas minor	−1.5° C	−0.80° C	0.70° C	Eliassen, Leivestad and Møller, 1960
FAMILY COTTIDAE				
Cottus scorpius	−1.5° C	−0.86° C	0.64° C	Eliassen, Leivestad and Møller, 1960
Gymnacanthus tricuspis	−1.73° C	−0.93° C	0.80° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
Icelus spatula	−1.73° C	−0.96° C	0.77° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
Myoxocephalus scorpius	−1.73° C	−1.44° C	0.29° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
	−1.7° C	−1.25° C	0.45° C	Gordon, Amdur and Scholander, 1962
FAMILY CYCLOPTERIDAE				
Cyclopterus lumpus	−1.5° C	−0.88° C	0.62° C	Eliassen, Leivestad and Møller, 1960
Liparis koefoedi	−1.73° C	−0.91° C	0.82° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
Liparis sp.	−1.9° C	−0.92° C	0.98° C	DeVries, 1970
FAMILY CYPRINODONTIDAE				
Fundulus heteroclitus	−1.5° C	−0.80° C	0.70° C	Umminger, 1969a
FAMILY GADIDAE				
Boreogadus saida	−1.73° C	−1.02° C	0.71° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
Gadus morhua	−1.5° C	−0.80° C	0.70° C	Eliassen, Leivestad and Møller, 1960
	−1.4° C	−0.76° C	0.64° C	Leivestad, 1965
Gadus ogac	−1.73° C	−1.47° C	0.26° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
	−1.7° C	−0.94° C	0.76° C	Gordon, Amdur and Scholander, 1962
Microgadus tomcod	−1.5° C	−0.98° C	0.52° C	Gordon, Amdur and Scholander, 1962
FAMILY NOTOTHENIIDAE				
Notothenia larseni	−1.87° C	−1.51° C	0.36° C	DeVries and Wohlschlag, 1969
Notothenia neglecta	−1.9° C	−1.08° C	0.82° C	Smith, 1968

TABLE III.—(*Continued*)

Family and species	Water temperature	Freezing point of serum	Amount serum supercooled	Reference
Notothenia rossii	−1.9° C	−1.07° C	0.83° C	Smith, 1968
Trematomus bernacchii	−1.73° C	−1.95° C	0° C*	Potts and Morris, 1968
	−1.9° C	−1.98° C	0°C*	DeVries, 1970
Trematomus borchgrevinki	−1.9° C	−2.07° C	0° C*	DeVries, 1970
Trematomus hansoni	−1.9° C	−2.01° C	0° C*	DeVries, 1970
Trematomus loennbergi	−1.9° C	−1.83° C	0.07° C	De Vries, 1970
Trematomus newnesi	−1.8° C	−1.01° C	0.79° C	Smith, 1968
FAMILY PLEURONECTIDAE				
Drepanopsetta platessoides	−1.5° C	−0.93° C	0.57° C	Eliassen, Leivestad and Møller, 1960
Pseudopleuronectes americanus	−0.80° C	−1.15° C	0° C*	Pearcy, 1961
	−1.0° C	−0.71° C	0.29° C	present paper
	−1.5° C	−0.78° C	0.72° C	present paper
FAMILY ZOARCIDAE				
Lycodes turneri	−1.73° C	−0.97° C	0.76° C	Scholander, van Dam, Kanwisher, Hammel and Gordon, 1957
Rhigophilia dearborni	−1.9° C	−1.52° C	0.38° C	DeVries, 1970

* Antifreeze present; freezing point of serum lower than temperature of water.

RESULTS

As indicated in Table I, the average serum osmolality of specimens of *P. americanus* acclimated to −1° C was not significantly different from the serum osmolality of fish acclimated to 15° C. The serum osmolality of flounder at −1° C was 388 mOsm/liter which corresponds to a serum freezing point of −0.71° C. These fish formed no "antifreeze," being supercooled by 0.29° C. Similarly, the serum osmolality of a single fish held at −1.5° C was 420 mOsm/liter; this fish was supercooled by 0.72° C. A single fish was placed into water at −1° C and the temperature was gradually lowered to −1.8° C over a seven-day period. At this temperature, ice began to form at the surface of the sea water and tiny crystals of ice began to circulate throughout the water in the aquarium. The presence of these crystals of ice caused the fish to freeze solid and die; presumably, the ice crystals seeded the supercooled blood, causing it to freeze.

Studies on freshly caught flounder (Table II) at 15° C showed that serum osmolality increased from 412 mOsm/liter in fish killed 1–2 hours after capture to 461 mOsm/liter in fish killed 5–6 hours after capture. Not only did serum osmolality increase significantly with time after capture, but also the serum osmolality of the freshly caught fish at 15° C (437 mOsm/liter) was significantly higher than the serum osmolality of fish acclimated to the laboratory at 15° C (389 mOsm/liter).

DISCUSSION

Pearcy (1961) found that specimens of *P. americanus* living in the Mystic River estuary in Connecticut during the winter of 1958–59 often encountered temperatures as low as –0.8° C. These winter fish had an average serum freezing point of –1.15° C. In contrast, the serum freezing point for fish collected in the summer was –0.63° C. In the present investigation, the serum freezing point (–0.71° C) of cold-acclimated winter fish is more similar to the summer than to the winter values reported by Pearcy (1961). What, then, accounts for the discrepancy in the data from these two studies?

The only obvious difference in the procedures used in the two investigations concerns the handling and capture of the fish. Pearcy (1962) used freshly caught flounder that may well have been stressed by the trawling, whereas the present investigation deals with fish that had been acclimated to the laboratory for several weeks. The present study shows that capture by trawl can elevate the serum osmolality of *P. americanus*. Therefore, the trawling procedures employed by Pearcy (1961) may account for the exceedingly high values he obtained for serum osmolality in winter fish. Similar studies by Slicher, Pickford and Pang (1966) on *Fundulus heteroclitus* showed that the serum osmolality was elevated in fish unaccustomed to handling when compared with fish "trained" to be familiar with the handling procedures. Therefore, Pearcy's account of "antifreeze" formation in the winter flounder at low temperatures was probably due to an increased serum osmolality produced by trawling stress during capture.

Another possible reason for the discrepancy in these two studies is that there may be yearly variations in the ability of the flounder to produce its "antifreeze." For example, Gordon, Amdur and Scholander (1962) were unable to find the high serum osmolalities reported by Scholander, van Dam, Kanwisher, Hammel and Gordon (1957) in two species of arctic fish. Gordon, Amdur and Scholander (1962) concluded that the difference in the serum osmolality measured during different years was real and that the amount of "antifreeze" added to the blood during the winter was extremely variable from year to year. This may well be the case with the winter flounder also.

Pearcy (1961) also found that winter flounder kept in the laboratory would freeze and die at temperatures between –1.0° C and –1.5° C. These lethally low temperatures were similar to the serum freezing points of freshly captured winter fish. Pearcy concluded that the flounder could not survive temperatures much below –1.15° C since this was the freezing point of the serum. Apparently, these data show that winter flounder cannot exist in a supercooled state. However, the present study shows that the winter flounder definitely can survive in a supercooled state. The fish freeze and die only when ice crystals are present in the water to nucleate the supercooled blood. In nature, surface ice is probably seldom encountered because winter flounder are bottom fish that often cover themselves with mud or sand. The ability of the winter flounder to avoid freezing by existing in a supercooled state (as reported in this paper) is the same mechanism utilized by the majority of cold-hardy marine teleosts (Table III) in surviving the subzero cold.

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Summary

1. The average serum osmolality of winter flounder, *Pseudopleuronectes americanus,* acclimated in the laboratory to −1° C (386 mOsm/liter) was not significantly different from the serum osmolality of fish at 15° C (389 mOsm/liter).

2. Winter flounder survived temperatures as low as −1.5° C in a supercooled state. When ice crystals were present in the sea water at −1.8° C, the fish froze and died due to nucleation of the supercooled blood.

3. The serum osmolality of freshly caught flounder increased significantly with time after capture (up to six hours) and was significantly higher than the serum osmolality of laboratory acclimated fish at the same temperature.

4. A previous account of "antifreeze" formation in the winter flounder at low temperatures was probably due to an increased serum osmolality produced by trawling stress during capture.

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